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**Ministry of
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MINISTRY OF ENVIRONMENT

IMPACT ASSESSMENT OF ACID MINE DRAINAGE
FROM EQUITY SILVER MINES
USING IN SITU BIOASSAY ANALYSIS

by

D. Bruce Maclean*
Bruce Maclean Environmental Services
R. R. #1
Telkwa, British Columbia
V0J 2X0

* formerly with
Ministry of Environment
Waste Management Branch
Smithers, British Columbia

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ABSTRACT

Maclean, D.B. 1988. Impact Assessment of Acid Mine Drainage from Equity Silver Mines Using In Situ Bioassay Analysis.

Investigations of the effects of acid mine drainage (AMD) from Equity Silver Mines near Houston, British Columbia, have included: water quality sampling, in situ bioassays using steelhead trout eggs and steelhead trout underyearlings as test organisms, and laboratory flow through bioassays. In situ bioassays were timed to run during the annual spring peak in ambient metal concentrations. Laboratory bioassays were performed to determine the LC50 of the AMD and to determine if the egg stage of development was more or less sensitive to metal toxicity than underyearlings. Results demonstrated that there was no in situ mortality in steelhead trout eggs or steelhead trout underyearlings with the improved ambient water quality present during April and May, 1987. A comparison with similar AMD bioassay work near Mt. Washington on Vancouver Island showed that large variations in copper toxicity occur with variable pH and hardness. Laboratory bioassay analysis showed toxicity occurred at AMD concentrations two to three times greater than previous studies had indicated. Recommendations are made for additional impact assessments using in situ and laboratory bioassay analytical methods.

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TABLE OF CONTENTS

	Page
Abstract	i
Acknowledgements	i
Table of Contents	ii
List of Figures	iii
List of Tables	iv
1 Introduction	1
1.0 Location of Study Area	1
1.1 Minesite Description	1
1.2 Acid Mine Drainage and Site Hydrology	1
1.3 Fisheries Values and Historical Toxicity	3
1.4 Objectives	7
2 Methods	7
2.1 Sample Points	7
2.2 <u>In Situ</u> Steelhead Egg Bioassay	8
2.3 <u>In Situ</u> Steelhead Underyearling Bioassay	9
2.4 Laboratory Egg and Sacfry Bioassays	9
3 Discussion	10
3.1 Water Quality	10
3.2 <u>In Situ</u> Steelhead Egg Bioassay	13
3.3 <u>In Situ</u> Steelhead Underyearling Bioassay	15
3.4 Laboratory Egg and Sacfry Bioassays	17
4 Summary and Recommendations	18
5 Literature Cited	21

LIST OF FIGURES

Figure		Page
1	<u>In Situ</u> Bioassay Locations in Bessemer Creek and Buck Creek Drainages	2
2	Mean Monthly Flow Hydrograph (Indexed) for the Buck Creek/Bessemer Creek Drainage	4
3	Dissolved Copper Concentrations at Bessemer Creek Site 0700081 - 1982-1987	5
4	Dissolved Zinc Concentrations at Bessemer Creek Site 0700081 - 1982-1987	6

LIST OF TABLES

Table	Page
1 Water Quality Variables Sampled During the 1987 <u>In Situ</u> Bioassay Testing	23
2 Non-Cumulative Log of Egg Mortality and Alevin Hatching During the <u>In Situ</u> Bioassay	24
3 Total Metal Concentration in Steelhead Trout Eggs and Alevins from the <u>In Situ</u> Bioassay	25
4 <u>In Situ</u> Bioassay Results for Steelhead Underyearling Trout	26
5 Total Metal Concentrations in Whole Steelhead Trout from the 23-Day <u>In Situ</u> Bioassay	26
6 Dissolved Metal Concentrations Present in Bioassay Toxicity Tests on Equity Silver Mines Acid Mine Drainage	27
7 Metal Concentrations in Steelhead Trout Alevin Tissue from the Flow Through Egg Bioassay	27
8 Bioassay Results from Laboratory Toxicity Testing	28

1. INTRODUCTION

1.0 Location of Study Area

Equity Silver Mines, located in the central interior of British Columbia approximately 35 km southeast of the District of Houston, began mining operations in 1980. The minesite situated at an elevation of 1275 metres straddles the headwaters of two watersheds; the Bessemer Creek/Buck Creek system south of the minesite and the Foxy Creek system to the north (Figure 1). The in situ bioassay work during this study was done in the Bessemer Creek/Buck Creek system. Drainages, permitted discharges, and uncontrolled seepages from the minesite pit areas enter the Buck Creek system upstream of Goosly Lake by way of Bessemer Creek. Buck Creek joins the Bulkley River at Houston approximately 55 km to the northwest.

1.1 Minesite Description

Silver, copper, and gold mineralization occurs in three distinct pyritic orebodies on the minesite. These are called the Main zone, Waterline zone and Southern Tail zone (Figure 1). Bessemer Creek originates immediately east of the pits and originally flowed through the Main zone pit area. During pit development, Bessemer Creek was diverted to prevent water entering the pit area. Getty Creek flowing from the south end of the Southern zone joins Bessemer Creek enroute to Buck Creek. Mining began in April 1980 in the Southern tail orebody and in March 1983 in the Main zone. Waterline zone is scheduled for development in 1988. Investigations beginning in November, 1981 revealed that seepages from the waste rock piles were acidic and contained elevated concentrations of metals. A collection and treatment system was installed during the spring of 1982.

1.2 Acid Mine Drainage and Site Hydrology

Acid Mine Drainage (AMD) which forms from the oxidation of pyritic waste rock (enhanced through bacterial action) has been a major cause of water quality problems at Equity Silver Mines since its discovery in 1981. The AMD process results in lowered pH values and very high concentrations of toxic metals in

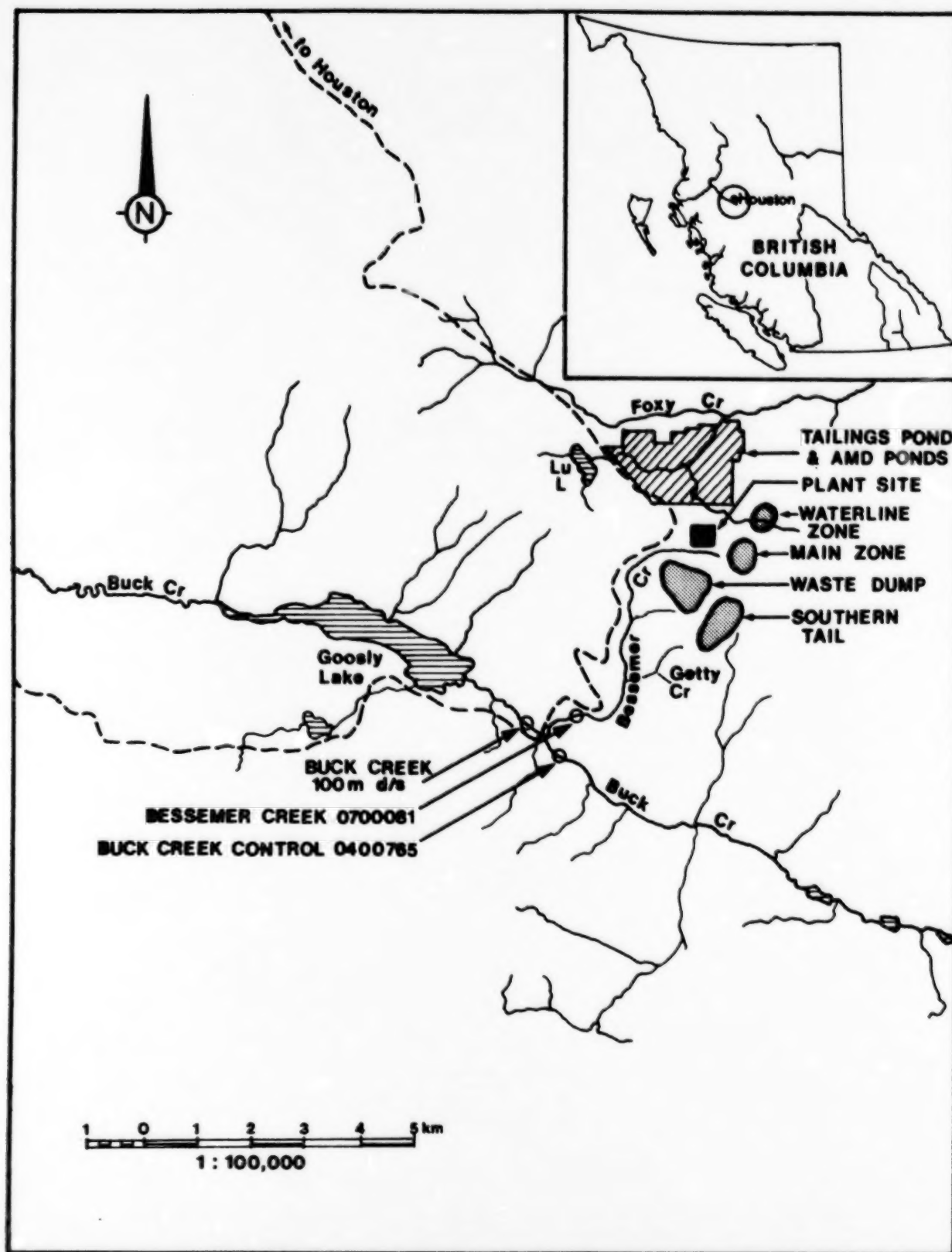


Figure 1. In Situ Bioassay Sites in Bessemer and Buck Creek Drainages

water that seeps through disturbed rocks at the minesite. Typical springtime AMD from Equity has the following characteristics: pH = 2.5, sulphate = 9000 mg/l, total Cu > 500 mg/l, total Zn > 200 mg/l, total Cd > 1.5 mg/l, total Al > 1500 mg/l. Equity Silver Mine generates approximately 800,000 m³ of AMD per year. Almost all of the AMD is now collected in ditches, treated to remove metals, and then discharged either to the Bessemer/Buck Creek drainage or to the Foxy Creek drainage during high water flows. However, AMD from undetected seepages causes significant increases in metal concentrations in the Bessemer/Buck Creek system.

These increases in metal concentrations are most noticeable in the early spring (April/May) when seasonal creek flow is still low but the initial spring snow melt has left the waste rock piles. The distribution of stream flow for the watershed is shown in Figure 2 along with the AMD collection system pump records (after Kerr Priestman & Associates, 1983 and Equity Annual Reclamation Report, 1987). The hydrograph shows that the peak flow for AMD occurs in April and early May when the stream flow has only increased slightly from winter low flows. These flow characteristics shown in the hydrograph result in a marked cyclical effect of metal concentrations in the Bessemer/Buck system. A summary of Equity's water quality monitoring for dissolved copper and dissolved zinc at Bessemer Creek for the period 1982 to 1987, shown in Figures 3 and 4 respectively, illustrates the cyclical pattern (Patterson 1988). These figures show the early spring peak metal concentrations that occur in Bessemer Creek. Notable also is the decline in peak values over time as AMD control measures were upgraded on the minesite.

1.3 Fisheries Values and Historical Toxicity

Fisheries values in the streams affected by Equity Silver Mine are summarized in the Norecol, 1986 and Bustard, 1987 reports.

Fish sampling upstream of Goosly Lake for tissue metal analysis showed the presence of rainbow trout, longnose suckers, reidsided shiners and prickly sculpins as resident species (Bustard 1984). No resident adult rainbows were found upstream of Goosly Lake during 1987, and juvenile rainbow trout found are presumed to be the progeny of Goosly Lake adults.

In general the habitat in Buck Creek between Goosly Lake and Bessemer Creek is poor for juvenile rainbow trout rearing while good spawning habitat exists

Figure 2. Mean Monthly Flow Hydrograph (Indexed) for the Bessemer and Buck Creek Drainages (after Kerr Priestman & Associates and Equity Silver Mines Ltd. Annual Report 1987)

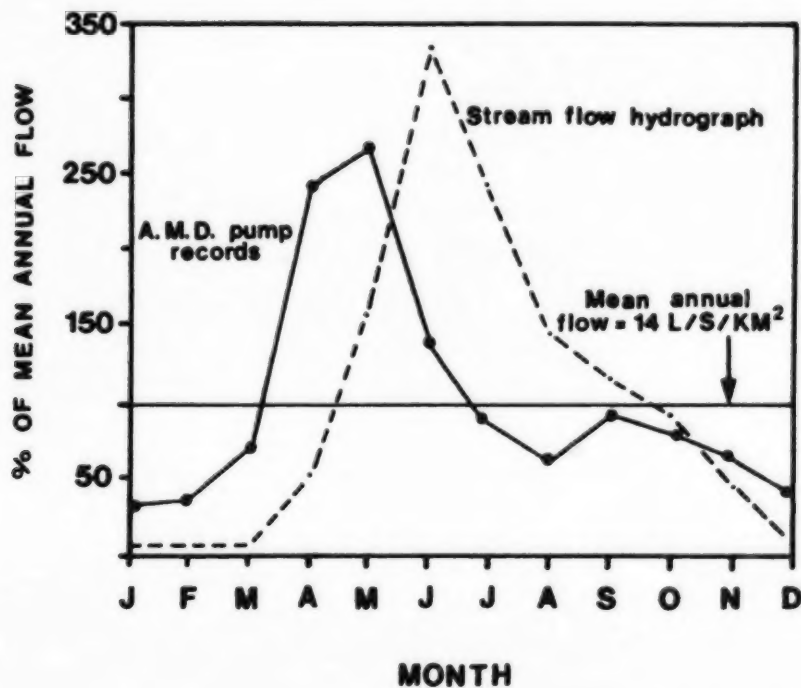


Figure 3. Dissolved Copper Concentrations at Bessemer Creek Site 07000081,
1982 - 1987

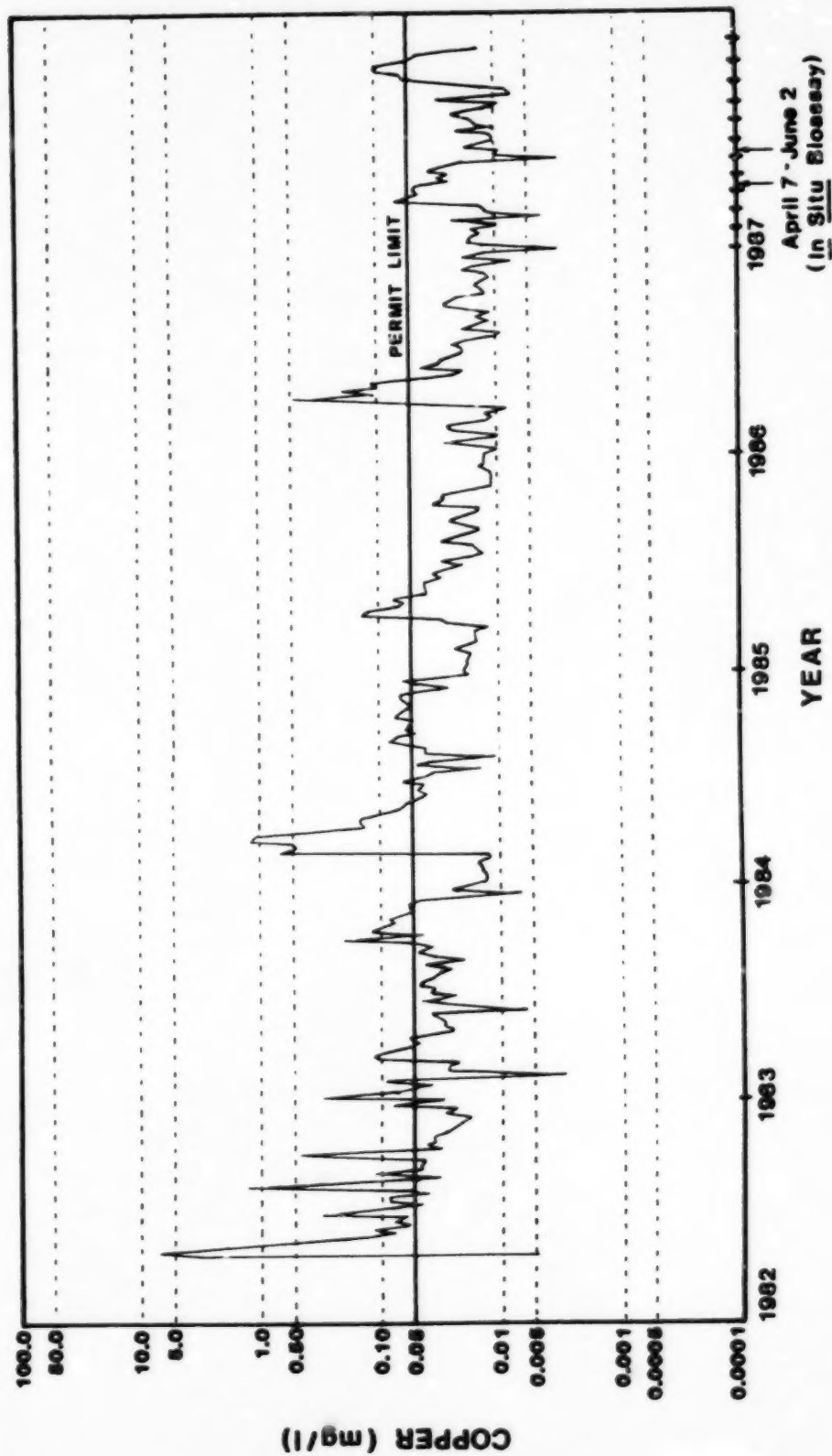
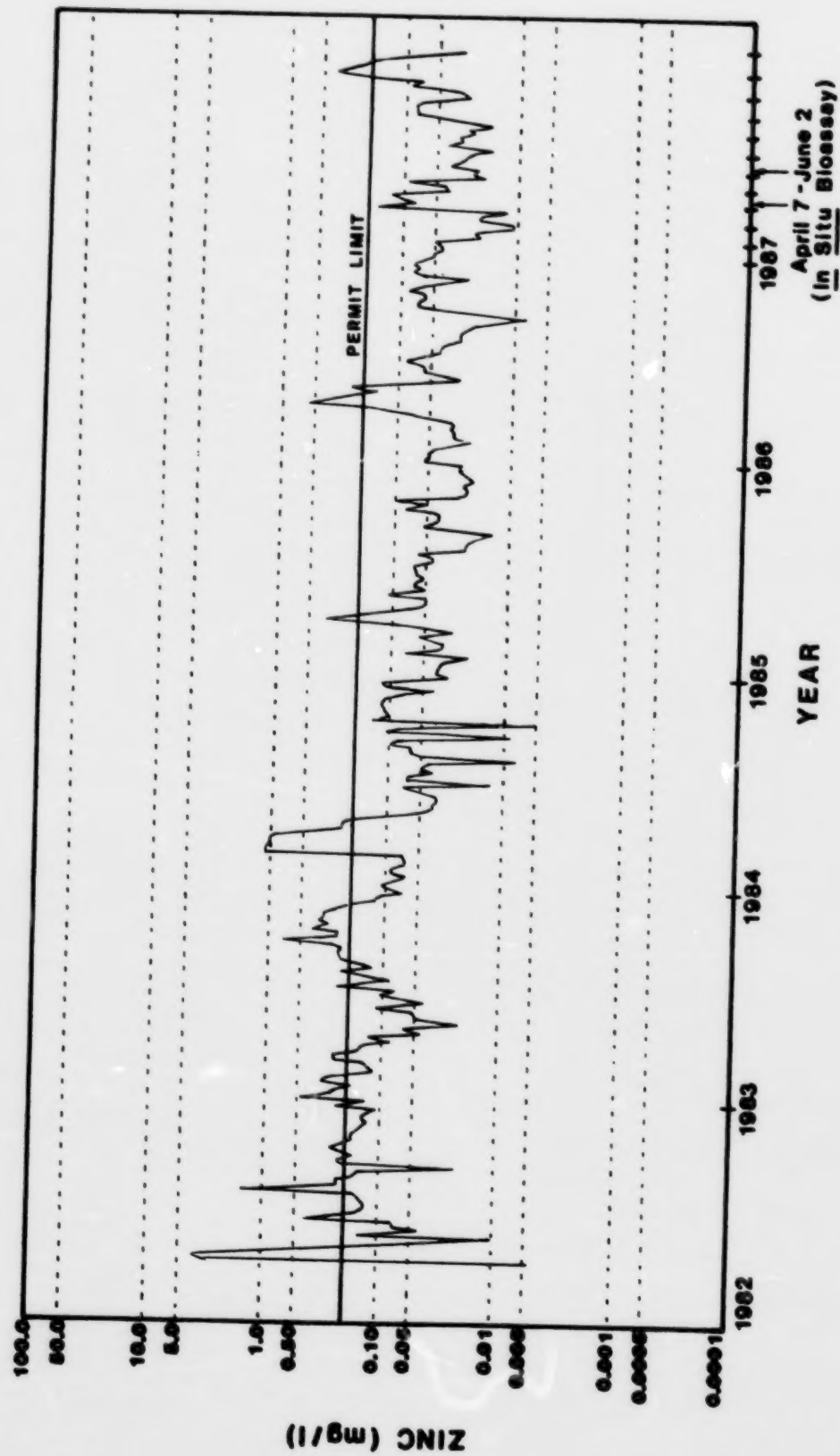


Figure 4. Dissolved Zinc Concentrations at Bessemer Creek Site 07000081,
1982 - 1987



above the Bessemer Creek confluence. Although suitable spawning or fry rearing habitat is rare between Goosly Lake and the Bessemer Creek confluence, rainbow parr are found in low density (Bustard 1987).

Standard bioassay methods (96 hour pass/fail) have shown that Bessemer Creek water is sometimes toxic to rainbow trout during the spring peak in metals concentration. Acutely lethal effects (failed 96 hour LC50) have been found only once on March 20, 1984 when values for dissolved Cu, Zn and Cd were .48 mg/l, 1.09 mg/l, and .0045 mg/l respectively. Bioassay toxicity work is summarized in Norecol's 1986 report.

1.4 Objectives

The objectives of the study were:

- (1) To determine the toxicity of Bessemer Creek and Buck Creek waters during the 1987 spring peak in metal concentrations using in situ bioassay methods. Steelhead trout underyearlings were used as the aquatic testing species. Steelhead trout eggs were used to determine whether earlier stages in the life cycle were more or less sensitive to the metal toxicants present in the creek's water and to determine if chronic or sublethal toxicity was occurring.
- (2) To determine what concentration of AMD is toxic to salmonid eggs and sac fry under laboratory flow through bioassay testing.

2. METHODS

2.1 Sample Points

Three sample points were selected for placing the bioassay test organisms.

- (1) Bessemer Creek just below the siltcheck dam. Water quality site #0700081. Under Waste Management Branch Permit PE-4475 this is considered a point of discharge for Equity Silver Mines. Water quality data for this site is extensive as shown in Figures 3 and 4.

- (2) Buck Creek above the Bessemer Creek confluence. Water quality site #0400765. This is the control site upstream of any mine influence on water quality.
- (3) Buck Creek 100 metres downstream of the Bessemer Creek confluence. This is the point where the receiving environment affected by Equity Silver Mine has aquatic resources influenced by the discharges from the mine-site.

2.2 In Situ Steelhead Egg Bioassay

Eyed steelhead trout eggs were obtained from the Abbotsford hatchery on April 6, 1987 and flown to Smithers on the same day. The eyed stage (when the eye spot in the egg is visible to the unaided eye) is preferred for in situ bioassay work as the eggs are hardy at this stage and can be transported without excessive mortality. Upon arrival in Smithers the eggs were sorted and any dead eggs destroyed; 150 eggs were placed in disinfected Whitlock-Vibert egg cases. The egg cases had been modified by gluing plastic netting on the lower chamber to prevent alevins from escaping. Due to the lack of good spawning gravel at the three selected sites it was necessary to construct artificial redds. These were constructed using a 30 cm square plywood base and chicken wire for the sides and top. 40 mm washed drain rock was placed in each wire cage to cover the egg cases to a depth of 10-15 cm. Three artificial redds were placed at each of the three sites: Bessemer Creek below the siltcheck dam, Buck Creek below the Bessemer Creek confluence, and Buck Creek above the Bessemer Creek confluence (control site). The redds were checked weekly for the presence of dead eggs and simultaneous weekly water samples were taken for metal and other water quality analysis. Water quality sampling results are shown in Table 1. One redd was designated as the checking redd to minimize disturbance to the eggs in the other replicates. A log of non-cumulative egg mortality is given in Table 2. At day 23 of the bioassay the checking redd was removed and the eggs frozen for subsequent metal accumulation analysis. The flow in Buck Creek at this point in time was approaching its spring maximum, but the remaining redds were left in place until day 57 to determine any sublethal effects on egg development.

At day 57 of the bioassay any remaining eggs and alevins were removed and frozen for subsequent metal accumulation analysis. Analytical results of metal concentrations in eggs and alevins are shown in Table 3.

2.3 In Situ Steelhead Underyearling Bioassay

At each of the three test sites 12 steelhead underyearlings between 30 and 50 mm in fork length were placed in stainless steel cages. Each cage was weighted with a rock of approximately 1 kg and placed on the creek bed where there was adequate depth and good flow. The cages were checked weekly and any dead fish were removed and frozen for subsequent metals analysis in tissue. All remaining live fish were killed on day 23 of the bioassay and frozen for metals analysis. Bioassay results are shown in Table 4. Due to the small size of the fish, separate liver and muscle tissue sampling could not be done. Analytical results of metal concentration in whole trout are given in Table 5.

2.4 Laboratory Egg and Sac Fry Bioassays

Steelhead trout eggs were placed in vertical incubation trays adapted for use in the flow through bioassay system used at the Provincial Ministry of Environment's Aquatic Toxicology Laboratory in North Vancouver. A detailed description of the flow through bioassay procedure is presented elsewhere (van Aggelen 1987). As the Aquatic Toxicology Laboratory supply water is of very low hardness the water was adjusted to a hardness of 45 mg/l as CaCO_3 which approximates the hardness of Buck Creek water. Previous standard bioassay work using juvenile rainbow trout as the test species indicated that the 96 hour LC_{50} of the AMD should be between 0.027 and 0.079 mg/l dissolved copper when the AMD is diluted in laboratory water of unadjusted hardness and pH6 or is diluted in Bulkley River water of 50 mg/l hardness and pH7.5 respectively (Wilkes 1987). Other bioassay work with Bessemer Creek water from site #0700081 had shown no acute lethality with 0.20 mg/l dissolved copper, 0.80 mg/l dissolved zinc, and 0.0022 mg/l dissolved cadmium, and pH7.7. Bessemer Creek water had shown acute lethality on March 20, 1984 with concentrations of 0.480 mg/l dissolved copper, 1.090 mg/l dissolved zinc 0.0045 mg/l dissolved cadmium, and pH6.4 (Norecol 1986). These previous bioassay results were used in the determination of concentrations used for the flow through bioassay tests. Concentrations of AMD used and the resulting diluted metal concentrations are shown in Table 6.

The initial concentrations of 120 ppm and 160 ppm AMD (.059 mg/l and .078 mg/l dissolved copper respectively) which were thought high enough to show toxicity, proved to be too low in concentration to show any toxic effects. The steelhead trout alevins that hatched from this initial bioassay were analyzed for metal concentrations in whole alevins. Results are shown in Table 7.

Additional LC50 bioassay work was done using the steelhead sac fry from the control group of the steelhead trout egg test. Two additional concentrations of 300 ppm and 400 ppm AMD (.147 mg/l and .196 mg/ dissolved copper respectively) were tested in the flow through bioassay system using rainbow trout eggs as the test organism. An additional 96 hour LC50 bioassay was done using the rainbow trout sac fry from the control group of the rainbow trout bioassay. Laboratory bioassay results are summarized in Table 8.

3. DISCUSSION

3.1 Water Quality

The water quality data presented in Table 1 from samples taken in Bessemer and Buck Creeks over the course of the in situ bioassays shows the elevation of metal concentrations in Bessemer Creek (0700081) and Buck Creek below the Bessemer confluence compared to the control site on Buck Creek (0400765). Bessemer Creek, however, did not have the high concentrations of copper and zinc seen at approximately the same time in previous years. Figures 3 and 4 show a steady decline since 1982 in annual spring peak values for dissolved copper and dissolved zinc respectively. Peak metal concentrations measured during the course of the in situ bioassay work occurred at Bessemer Creek on the first day of the bioassay and concentrations declined from day 1. During the course of the in situ bioassay treated AMD was discharged by Equity from April 14 to May 25.

Dissolved copper exceeded the Waste Management Branch permitted level of 0.05 mg/l in Bessemer Creek on two occasions during the sample period; .09 mg/l on April 8 and .06 mg/l on April 13. Copper concentrations in Bessemer Creek declined during the period of permitted treated AMD discharge as creek flow

increased during freshet. Equity Silver Mines monitoring indicates no further excursions for dissolved copper. The recommended guidelines for total copper for receiving waters of different hardnesses are as follows (CCREM, 1987):

Hardness (mg/l as CaCO ₃)	Concentration of total Copper (mg/l)
0 - 60	.002
60 - 120	.002
120 - 180	.004
> 180	.006

As shown in Table 1 total copper equaled or exceeded the criteria at all three sites on all sampling occasions. Bessemer Creek's influence on water quality in Buck Creek 100 m downstream of the confluence compared to the Buck Creek above Bessemer Creek site shows a significant increase in total copper concentrations ($p < .01$ $n=8$) during the course of the in situ bioassay.

Dissolved zinc did not exceed the Waste Management Branch permitted level of 0.20 mg/l during the sample period. The water quality criteria for total zinc is set at .03 mg/l (CCREM 1987). The recommended guidelines for total zinc concentrations in receiving waters of different hardnesses are as follows (Taylor 1980):

Hardness (mg/l as CaCO ₃)	Concentration of total Zinc (mg/l)
0 - 120	0.05
120 - 180	0.10
180 - 300	0.20
> 300	0.30

Bessemer Creek exceeded the criteria for the first sample while the two Buck Creek sites never exceeded the criteria.

The water quality criteria for total lead is set at .005 mg/l at a water hardness of < 95 mg/l and .01 mg/l at a water hardness of >95 mg/l (Demayo 1980). The Buck Creek control site was at the criteria level on two occasions while the Buck Creek 100 m downstream site was below the criteria in all the water quality samples.

The high concentration of total lead on May 28 at Bessemer Creek is not explainable and is probably an anomalous value.

The tentative water quality criteria for total aluminum in waters with pH greater than 6.5 is set at 0.1 mg/l (CCREM, 1987). Table 1 shows that all 3 sites exceeded this recommendation for all samples.

The water quality criteria for total arsenic is set at 0.05 mg/l (CCREM, 1987). All the water samples taken during the course of the bioassay were within the recommended criteria.

Total cadmium was measurable (above laboratory detection limits) only on the first day of the bioassays and only at Bessemer Creek.

Total residue was high in Bessemer Creek and elevated in Buck Creek below Bessemer Creek. The high total residue concentrations paralleled the occurrence of sedimentation in the Whitlock-Vibert cases after day 29 of the bioassay. Water hardness and sulfate concentrations were elevated in Bessemer Creek and Buck Creek downstream of Bessemer Creek compared to Buck Creek control concentrations. Sulfate and hardness are elevated in Bessemer Creek by the discharge of treated AMD from the minesite to Bessemer Creek (treated AMD contains approximately 1500 mg/l sulfate). As will be discussed in Section 3.2, this increase in hardness has a direct effect on the toxicity of metals to aquatic life.

The pH values were slightly alkaline during the course of the bioassay with a mean value of 7.4 units.

3.2 In Situ Steelhead Egg Bioassay

The non-cumulative mortality as shown in Table 2 indicates that no acutely toxic effects occurred to the eggs during the course of the in situ bioassay. As will be discussed later, the laboratory flow through egg bioassay began to show toxicity to eggs at an AMD concentration of 400 ppm at water hardness of 45 mg/l (corresponds to .196 mg/l dissolved Cu). Bessemer Creek water sampling showed a maximum copper concentration of 0.09 mg/l April 8, 1987.

It is assumed that the toxicity of metals to steelhead eggs is greatly affected by hardness as for other aquatic life, increasing hardness being associated with reduced toxicity.

Copper toxicity to rainbow trout underyearlings is variable depending on water hardness and pH. The table below (after Howarth 1977) shows the median lethal concentrations of dissolved copper to rainbow trout with varying hardness and pH;

Average Total Hardness mg/l	Average pH	96 hr LC50 Cu mg/l
32	6.0	.022
31	8.0	.030
101	6.0	.040
100	7.0	.048
366	6.0	.070
361	7.0	.298
371	8.0	.516

These bioassay results, although using copper alone as the sole toxic metal, show the variable effect that hardness and pH have on the toxicity of copper to rainbow trout underyearlings. No references can be found in the literature showing variable effects of water hardness and pH on copper toxicity to fish eggs. Embryo mortality occurred in rainbow trout eggs at a

copper concentration of 0.037 mg/l (Demayo 1981). Research in this area also indicates that rainbow trout embryos are affected by teratogenesis as much as by straight mortality when exposed to copper concentrations as low as .001 - .005 mg/l. Teratogenic effects were mainly skeletal deformations such as scoliosis and kyphosis (Demayo, 1981).

While peak metal concentrations declined through the course of the in situ egg bioassay, the eggs were allowed to develop after day 29 to determine if any sublethal effects would occur on egg development. Partial hatching of alevins with only the head and yolk protruding from the egg capsule has been observed in pink and sockeye salmon exposed to .055 mg/l dissolved copper and 83.1 mg/l hardness (Servizi 1978). Impaired survival, growth and development have been reported in the development of rainbow trout eggs to the fingerling stage when their exposure to total copper is at 0.01 mg/l (Demayo 1981). The accumulation of sediment in the Whitlock-Vibert egg cases resulted in the maceration and mortality of a large proportion of developing eggs and alevins so a large part of this aspect of the in situ bioassay was lost. The shape and development of surviving steelhead alevins were examined under a hand lens. Alevins appeared to be normal at all three sites.

Analysis of the total metal concentrations in the steelhead trout eggs shows that copper is significantly elevated in eggs from the Bessemer Creek site when compared to the Buck Creek control site ($p < .01$ $n=3$) but copper is not significantly elevated in eggs from Buck Creek downstream of the Bessemer confluence compared to the Buck Creek control site. Zinc is not significantly elevated at the Bessemer Creek site or the Buck Creek downstream site when compared to the Buck Creek control ($p > .01$ $n=3$).

Similar continuous in situ bioassay work was done during May and June 1986 at an abandoned copper mine on Mt. Washington, Vancouver Island. This study showed severely toxic conditions occurring in the creeks affected by the uncontrolled discharge of AMD (Erickson 1986). The results were as follows:

Site	Mortality	pH mean	Hardness mg/l mean	Total Dissolved Cu mg/l			
				M 13	M 21	M 27	Jn 3
Pyrrhotite Creek	100%	5.8	12.4	.17	.58	.22	.25
Murex Creek	81%	7.0	8.0	.03	.09	.08	.10
Tsolum River	16%	7.0	10.6	.04	.03	.03	.03
McKay Creek	12%	-	-	.003	.002	.009	.02
Upper Murex Creek	12%	-	-	.011	.005	-	-

AMD (copper) from the Mt. Washington mine seeps mainly into Pyrrhotite Creek and then flows into Murex Creek and then into the Tsolum River with corresponding lowered concentrations. McKay Creek and Upper Murex Creek (which drain into Murex Creek) receive lessor amounts of the AMD.

A direct comparison of the results from the Mt. Washington continuous in situ egg bioassay with the results from the Equity Silver Mines drainage cannot realistically be made due to the different characteristics of the surface waters. Vancouver Island drainages tend to have surface water with characteristically very low hardness and slightly acid pH, while the surface waters around Equity Silver Mines are characterized as moderately hard and slightly alkaline pH. A comparison of the two bioassays emphasizes that large variations in copper toxicity occur with variable pH and hardness.

3.3 In Situ Steelhead Underyearling Bioassay

The use of in situ bioassay techniques is a proven method of determining the toxicity of water that is of erratic or variable quality since static bioassays are run with only an instantaneous sample of unknown water quality. In situ bioassay techniques do not seem applicable in determining liver or muscle tissue metal accumulation as the fish are limited in their exposure time due to feeding restraints in the cages. Also, as the caged fish are not actively feeding, the food chain sources of metals are not entirely accounted for.

Table 4 shows the results of the in situ bioassay which ran from April 7 to April 30, 1987. Three fish were found crushed under the rock weighting the

case. Two fish died after 23 days exposure at the Bessemer Creek site. Given the low level of copper and high level of hardness measured over the course of the in situ bioassay, it is believed that these fish probably died from other causes (possibly starvation stress). Previous bioassay work in Bessemer Creek has shown that copper, zinc, and cadmium concentrations of 0.20, 0.80 and 0.0032 mg/l respectively did not show acute lethality (Norecol 1986). Due to the small size of the fish separate liver and muscle tissue samples could not be taken for metal analysis; the fish were composited and whole fish were analyzed. The metal concentrations shown in Table 5 indicate that neither copper nor zinc from the Bessemer Creek site or Buck Creek downstream site are significantly elevated in concentration as compared to the Buck Creek control ($p > .01$, $n=2$).

Similar in situ bioassay work at Mt. Washington in the Tsolum River and Murex Creek drainages showed the following results:

Site	Mortality	Dissolved Cu (mg/l)			Avg. hardness (mg/l)
		May 27	June 3	June 9	
Murex Creek	100%	.08	.10	.06	8.6
Tsolum R 1 km below Murex Cr	80%	.03	.03	.02	11.3
McKay Cr	50%	.03	.03	.02	11.3
Tsolum R above Murex Cr	0%	.001	.002	.002	16.1

The 50% mortality at the Tsolum River below Murex Creek occurred during the first 9 days when dissolved copper levels were at .03 mg/l and hardness averaged 10.4 mg/l. 95% of the mortality in Murex Creek occurred during the first 6 days when dissolved copper levels were between .08 mg/l and .10 mg/l. Here again, as in the in situ egg bioassay, water hardness is very important in determining the toxicity of metals (copper). Dissolved copper values in Bessemer Creek during the in situ bioassay were between .04 mg/l and .09 mg/l while hardness was between 184 mg/l and 502 mg/l. The fact that no toxicity

occurred in Bessemer Creek can probably be explained by the additional hardness present in Bessemer Creek.

3.4 Laboratory Egg and Sacfry Bioassays

Table 8 summarizes the laboratory bioassay work using AMD collected on April 7, 1988 at Equity Silver Mines. The initial test concentrations of 120 ppm and 160 ppm AMD used in the steelhead egg bioassay showed no toxicity to the development of the embryos. Visual inspection of the alevins showed no obvious malformations. Metal concentrations analyzed in the alevins and presented in Table 7 show no pattern of metal accumulation in the fish.

Alevins from the control group of this bioassay were used as the test organism in a standard 96 hour LC50 acute toxicity determination. The acute toxicity was determined at a concentration of 460 ppm AMD which is equivalent to dissolved copper, zinc and cadmium concentrations of .22 mg/l, .101 mg/l and .0009 mg/l respectively. Hardness of the laboratory water was adjusted to 45 mg/l. The concentrations of metals required to cause acute toxicity in the alevins is surprisingly high. The 96 hour LC50 values for steelhead alevins exposed to dissolved copper alone in water of 24 mg/l hardness and pH 7.1 units was found to be .028 mg/l (Chapman 1978). Waste Management bioassay tests have indicated 96 hour LC50 values of .079 mg/l dissolved copper in water of 50 mg/l hardness. The work of Howarth et al (see abridged table in section 3.3) shows that the lethal response to dissolved copper is complex with varying pH and hardness combinations; however, based on copper toxicity alone, steelhead alevins would have been expected to show a lethal response between 100 ppm and 200 ppm AMD concentrations.

Following the initial tests using steelhead eggs, two additional concentrations of AMD were treated in laboratory water of adjusted hardness (45 mg/l) using rainbow trout eggs as the test organism. 300 ppm AMD showed no deleterious effects while 400 ppm AMD resulted in 15% egg mortality. 400 ppm AMD is equivalent to a dissolved copper concentration of .196 mg/l. A comparison to similar in situ mortality (16%) in the Tsolum River on Vancouver Island (see table in section 3.2) shows again the lower threshold of acute toxicity in water of low hardness.

The alevins from the control group of the rainbow trout egg bioassay were used in a standard 96 hour LC50 acute toxicity determination using hardness adjusted (45 mg/l) laboratory water. The 96 hour LC50 was determined to be 500 ppm of AMD which is equivalent to dissolved copper, zinc and cadmium concentrations of .245 mg/l, .110 mg/l and .001 mg/l respectively.

A final test using standard 96 hour LC50 acute toxicity methods in laboratory water which was not hardness adjusted (i.e. 5 mg/l) and using rainbow trout as the test organism resulted in an LC50 toxic concentration of 51 ppm of AMD equivalent to dissolved copper, zinc and cadmium concentrations of .025 mg/l, .011 mg/l and .0001 mg/l respectively. This copper value of .025 mg/l is very close to the 96 hour LC50 value of 0.027 mg/l dissolved copper (coincidentally also 51 ppm AMD) tested in February 1987 (Wilkes 1987).

4. SUMMARY AND RECOMMENDATIONS

The use of in situ bioassay methods is a sound ecological approach to the determination of the toxicity of pollutants to organisms in their natural environments. The in situ bioassay method can provide direct evidence regarding potential deleterious effects of the metal loadings in the receiving waters affected by Equity Silver Mines. The concentration of metals present in Buck Creek and Bessemer Creek during April 1987 was low enough that no acutely toxic effects were observed in the in situ steelhead eggs or the in situ steelhead trout underyearlings.

The laboratory toxicity work showed acute toxicity to steelhead alevins when the AMD is diluted to a dissolved copper concentration of .22 mg/l in water of 45 mg/l hardness. Previous Ministry of Environment bioassay work using AMD in laboratory water of unadjusted hardness (4.0 mg/l) indicated toxicity at copper concentrations 10 times lower (.027 mg/l) and when the AMD is diluted in Bulkley River water (hardness of 50.0 mg/l) the toxicity occurred at copper concentrations 2.8 times lower (.079 mg/l). The variability in AMD toxicity caused by varying hardness and pH demonstrates the value of in situ bioassays when water quality varies over a wide range of metal concentrations and hardness values.

Abundant work has been done to show experimentally these variations in toxicity of different metals to different species of salmonids (summarized in Alderice and McLean 1982). The toxicity of combinations of metals (copper, zinc, and cadmium) indicates that the toxicity of copper:zinc in high ratios (1:3) tends to be additive for the individual metals while the toxicity in low ratios (1:12) tend to be less than additive i.e. antagonistic (Finlayson 1982). All of these experiments have been done in water of relatively low hardness and in the case of combinations of metals in water with copper:zinc ratios that do not occur in the receiving waters around Equity Silver Mines. Equity AMD tends to a copper:zinc ratio of 1:0.5 while the receiving waters around Equity tend to a ratio of 1:1.5. As the experimental work to date cannot be realistically extrapolated to the Equity situation there is a need for further on-site toxicity determination.

In the Bessemer Creek/Buck Creek system the focus of in situ analysis should be on the life stage that occurs in the watershed and is impacted by changes in water quality from mining activity. Aquatic life is first impacted by the mine discharges in Buck Creek downstream of the Bessemer Creek confluence. Dave Bustard has found low densities of rainbow trout parr in the reaches of lower Buck Creek. However, there is a lack of good spawning habitat below the Bessemer Creek confluence and above Goosly Lake. Good spawning habitat is found only above the confluence of Bessemer Creek.

The Buck Creek 100 m below Bessemer Creek site had total copper concentrations in excess of the recommended guidelines for the protection of aquatic life on all sampling occasions and on April 7, 1987 with .03 mg/l total copper was approaching toxic concentrations. Concentrations of metals in Bessemer and Buck Creeks declined from day 1 of the in situ bioassay. Rainbow trout when exposed to pulses of near lethal copper showed a decrease in survival even though the average concentration was below the no-effect concentration (CCREM 1987).

In light of these facts additional in situ bioassays are recommended at the lower Buck Creek site using rainbow trout underyearlings as the test organism. Timing of the bioassay is recommended to be as early in the spring as ice conditions would allow. If the fish can be placed as early as mid-March then a second bioassay could be run subsequent to the first. Each in situ bioassay should not exceed 21 days in duration to avoid starvation stress on the fish.

Chau and Wong (1975) found in Babine Lake that waters with high dissolved organic content (as indicated by dissolved organic carbon) had high complexing capacity for copper. Complexation with humic acid has been shown to reduce acute and chronic toxicity of copper in bioassays with *Daphnia* (spp) (Winner 1985). Future toxicity studies could attempt to measure the complexing capacity of Buck Creek water or at least should include a measurement of organic carbon.

Due to the difficulty with sedimentation in the Whitlock-Vibert egg cases, a more elaborate in situ design would be necessary to determine the chronic effects on fish eggs of water quality changes in Bessemer Creek and Buck Creek. Suspended sediment could be removed from the creek water and the clarified water directed to a flow through system on site. However, because no spawning habitat is immediately affected by changes in water quality caused by the mine, further in situ egg bioassay work is not recommended.

Since spawning habitat downstream of Goosly Lake could eventually be affected by changes in water quality and because the threshold for egg mortality was found at high concentrations of dissolved copper (.196 mg/l) in hardness adjusted laboratory water, it is recommended that the laboratory bioassay be repeated using AMD diluted in Buck Creek water from downstream of Goosly Lake rather than hardness adjusted laboratory water. Data from the further work would be valuable in establishing safe water quality objectives for Buck Creek.

Further recommended toxicity work would include using the zooplankton *Daphnia* (spp) as the test organism. *Daphnia* (spp) are abundant in Goosly Lake and bioassay tests have shown that very low concentrations of copper can cause acute toxicity (.007 mg/l at 50 mg/l hardness) (CCREM 1987). Buck Creek 100 metres downstream of Bessemer Creek has copper concentrations in excess of these concentrations and could be impacting on the zooplankton community in the east end of Goosly Lake. Chronic toxicity analysis using *Daphnia* reproductive failure is also recommended.

Results from these additional toxicity studies would be valuable in assessing whether the Waste Management Branch's permitted discharge levels (.05 mg/l dissolved copper, .20 mg/l dissolved zinc) are stringent enough to protect aquatic life in waters downstream of Equity Silver Mines.

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TABLE 1
WATER QUALITY VARIABLES SAMPLED DURING THE 1987 IN SITU BIOASSAY TESTING

LOCATION	pH units	Spec Cond um/cm	Total Residue mg/l	Non-flit Residue mg/l	Sulfate mg/l	Hardness mg/l	Total Al mg/l	Diss Al mg/l	Total As mg/l	Diss As mg/l	Total Cu mg/l	Diss Cu mg/l	Total Zn mg/l	Diss Zn mg/l	Total Cd mg/l	Diss Cd mg/l	Total Fe mg/l	Diss Fe mg/l	Total Pb mg/l	Diss Pb mg/l
Buck Creek above Bessemer																				
0400765																				
87/04/07	7.8	125	108	2	4.1	73.1	.51	.18	.001	<.001	.007	.002	<.005	<.005	<.01	<.01	1.8	.72	.005	<.001
87/04/13	7.5	115	108	4	3.2	60.0	.37	.24	<.001	<.001	.003	.001	<.005	*	<.01	<.01	1.00	.67	.004	.001
87/04/21	7.6	85	114	10	3.6	56.1	.39	.24	.001	.001	.002	.001	<.005	<.005	<.0005	<.0005	.99	.58	.002	.001
87/04/30	7.4	61	96	5	3.7	45.4	.83	.34	<.001	<.001	.004	.002	<.005	<.005	<.0005	<.0005	1.28	.51	.003	.001
87/05/05	7.5	61	86	2	4.0	31.8	.54	.33	<.001	<.001	.003	.003	.005	.005	<.0005	<.0005	.74	.46	.003	.001
87/05/19	7.1	53	88	3	3.7	30.9	.43	.24	<.001	<.001	.004	.002	<.005	<.005	<.01	<.01	.44	.27	.005	<.001
87/05/28	7.3	63	88	3	3.0	24.1	.32	.21	<.001	<.001	.003	.002	<.005	<.005	<.01	<.01	.34	.31	.002	.001
87/06/02							.33	.19	<.001	<.001	.002	.001	<.005	<.005	<.01	<.01	.48	.28	.003	<.001
Buck Ck 100 m downstream of Bessemer																				
87/04/07	7.5	173	144	4	31	116	1.4	.29	.002	<.001	.03	.014	.03	.03	<.0005	<.0005	3.42	.62	.003	<.001
87/04/13	7.4	219	176	3	54.9	81.9	.47	.22	.002	<.001	.01	.005	.013	.013	<.01	<.01	1.27	.66	.004	<.001
87/04/21	7.3	248	248	30	80.5	103	.39	.25	.002	<.001	.005	.004	.007	.007	<.01	<.01	1.04	.62	.003	<.001
87/04/30	7.3	150	160	13	40.9	67.5	.87	.34	<.001	<.001	.016	.009	.02	.019	<.01	<.01	1.73	.38	.004	.003
87/05/05	7.3	328	260	4	113	143	.32	.32	.001	<.001	.006	.005	.008	.008	<.0005	<.0005	1.05	.41	.003	.002
87/05/19	7.0	87	104	7	15.0	46.5	.45	.20	<.001	<.001	.005	.003	.009	.008	<.01	<.01	.43	.24	.003	<.001
87/05/28	7.3	99	106	2	16.0	48.5	.31	.21	.001	.001	.004	.004	<.005	<.005	<.01	<.01	.55	.26	.002	<.001
87/06/02													<.005	<.005	<.01	<.01	.51	.30	.003	<.001
Bessemer Ck - 0700081																				
87/04/08	7.6	393	300	6	144	187	4.2	.47	.009	<.001	.25	.09	.30	.20	.0022	<.0005	3.45	.23	.008	<.001
87/04/13	7.3	1000	838	5	495	184	2.3	.36	.003	<.001	.11	.06	.13	.08	<.01	<.01	2.05	.18	.003	.001
87/04/21	7.2	912	850	55	451	502	1.2	.48	.002	<.001	.08	.04	.16	.13	<.01	<.01	1.00	.09	.002	<.001
87/04/30	7.3	1480	1370	9	828	474	3.26	.45	.005	<.001	.11	.05	.14	.10	<.01	<.01	3.44	.13	.004	.001
87/05/05	7.2	294	266	17	108	828	.92	.35	.002	<.001	.02	.02	.08	.07	<.01	<.01	.76	.06	.002	<.001
87/05/19	7.4	370	298	6	131	158	1.69	.26	.003	<.001	.03	.02	.03	.01	<.01	<.01	1.66	.14	.012	.001
87/05/28							1.01	.32	.002	<.001	.03	.02	.03	.02	<.01	<.01	1.10	.15	.002	<.001
87/06/02																				
Acid Mine Drainage																				
87/04/08						7150	1640			153	489		219		1.91		8450			1.5

TABLE 2

NON-CUMULATIVE LOG OF STEELHEAD EGG MORTALITY AND ALEVIN HATCHING DURING THE IN SITU BIOASSAY

	Bessemer Creek			Buck Creek d/s			Buck Creek Control		
	Redd 1	Redd 2	Redd 3	Redd 1	Redd 2	Redd 3	Redd 1	Redd 2	Redd 3
Day 1	Installed 150 eggs	Installed 150 eggs	Installed 150 eggs	Installed 150 eggs	Installed 150 eggs	Installed 150 eggs	Installed 150 eggs	Installed 150 eggs	Installed 150 eggs
Day 6	2 dead eggs	--	--	0 dead eggs	--	--	2 dead eggs	--	--
Day 14	0 dead eggs	--	--	0 dead eggs	--	--	1 dead egg	--	--
Day 23 *1	0 dead eggs	--	--	2 dead eggs	--	--	0 dead eggs	--	--
Day 29	--	2 dead eggs	--	--	5 dead eggs	--	--	--	--
Day 42 *2	--	1 dead egg	--	--	3 dead eggs	--	--	3 dead eggs	--
Day 51	--	5 dead eggs 11 alevins	--	--	--	--	--	--	--
Day 56	--	6 dead eggs 27 alevins *3	1 dead egg 6 alevins *3	--	30 dead eggs 105 alevins *3	76 dead eggs 25 dead alevins 15 live alevins *3	--	25 dead eggs 2 alevins	10 dead eggs 8 alevins
								2 dead eggs 78 alevins *3	10 dead eggs 3 unhatched 110 alevins *3

*1 1 Vibert box pulled from each site, trout removed for metals analysis

*2 Sedimentation in Vibert boxes

*3 Physical effect of siltation contributes to mortality. In the Bessemer Creek redds there was a silty organic residue which the writer concluded macerated eggs and alevins.

TABLE 3

TOTAL METAL CONCENTRATIONS IN STEELHEAD TROUT EGGS
AND ALEVINS FROM THE IN SITU BIOASSAY

(ug/g)

SITE/EXPOSURE TIME	% MOISTURE	Al	As	Ba	Cd	Cu	Mn	Pb	Zn
<u>Buck Creek Control</u>									
23 days - egg	63.8	8	<25	<1	<1	3	4	<10	33
52 days - eggs & alevins	61	1220	<25	82	<1	6	1060	<10	62
Rep 1 57 days - eggs & alevins	63.5	129	<30	8	<1	6	89	<10	72
Rep 2 57 days - eggs & alevins	68.1	29	<30	<1	<1	3	6	<10	76
Rep 3 57 days - eggs & alevins	63.4	374	<30	66	<1	4	1240	<10	68
<u>Buck Creek d/s Bessemer Confluence</u>									
23 days - egg	62.7	24	<25	<1	<1	9	5	<10	68
Rep 1 57 days - alevins	62.1	2340	<25	52	<1	24	573	<10	78
Rep 2 57 days - alevins	50.7	5080	<25	124	<1	56	866	<10	94
Rep 3 57 days - alevins	66.0	1430	<25	36	<1	12	284	<10	41
<u>Bessemer Creek</u>									
23 days - egg	64.2	22	<25	<1	<1	33	3	<10	69
52 days - alevins	65.2	1230	<25	15	<1	10	46	<10	82
Rep 4 57 days - alevins	64.8	1760	<25	24	<1	42	175	<10	112

TABLE 4

IN SITU BIOASSAY RESULTS FOR STEELHEAD UNDERYEARLING TROUT

SITE	# TEST FISH	FORK LENGTH AVERAGE (mm)	CUMULATIVE MORTALITY AT TIME (DAYS)			
			START	6d	14d	23d
Buck Ck Control	12	45	0	1*	2*	2*
Buck Ck d/s Bessemer Ck	12	45	0	0	0	1*
Bessemer Ck at siltcheck	12	45	0	0	0	2

* Fish apparently killed by rock weighting cage

TABLE 5

TOTAL METAL CONCENTRATIONS IN WHOLE STEELHEAD TROUT
FROM THE 23 DAY IN SITU BIOASSAY

(ug/g)

SITE	% MOISTURE	Al	As	Ba	Cd	Cu	Mn	Pb	Zn
BUCK CK CONTROL #0400765									
Replicate 1	80.2	113	<30	3	<1	6	27	<10	123
Replicate 2	89.6	96	<25	4	<1	8	35	<10	143
BUCK CK D/S BESSEMER CK									
Replicate 1	87.8	122	<25	5	<1	11	29	<10	206
Replicate 2	82.6	69	<25	3	<1	9	20	<10	175
BESSEMER CK #0700081									
Replicate 1 - 2 fish that	78.3	107	<25	<1	<1	10	14	<10	149
died <u>in situ</u>									
Replicate 2	83.7	76	<25	2	<1	11	11	<10	147
Replicate 3	80.4	136	<25	3	<1	16	16	<10	167

TABLE 6

DISSOLVED METAL CONCENTRATIONS PRESENT IN BIOASSAY
TOXICITY TESTS ON EQUITY SILVER MINES ACID MINE DRAINAGE
(mg/l)

	Al	Cu	Zn	As	Cd
AMD - undiluted	1640	489	219	153	191
51 ppm	.084	.025	.011	.008	.0001
120 ppm	.197	.059	.026	.018	.0002
160 ppm	.262	.078	.035	.025	.0003
300 ppm	.492	.147	.066	.045	.0006
400 ppm	.656	.196	.088	.061	.0008
460 ppm	.754	.22	.101	.070	.0009
500 ppm	.82	.245	.110	.077	.001

TABLE 7

METAL CONCENTRATIONS IN
STEELHEAD TROUT ALEVIN TISSUE FROM
THE FLOW THROUGH EGG BIOASSAY

(ug/g)

	% moisture	Al	As	Ca	Cd	Cu	Fe	Mg	Mn	Pb	Sr	Zn	P
Control	80.7	22	<25	4100	<1	5	45	1180	2	<10	9	80	11400
120 ppm AMD	79	24	<30	2750	<1	1	34	1290	2	<10	7	63	11500
160 ppm AMD	79.1	22	<30	2970	<1	2	33	1260	2	<10	6	68	11700

TABLE 8

BIOASSAY RESULTS FROM LABORATORY TOXICITY TESTING

TEST ORGANISM	CONCENTRATION OF AMD	HARDNESS (mg/l)	DISSOLVED CU (mg/l)	DISSOLVED ZN (mg/l)	RESULTS
steelhead trout eggs	control	4	-	-	no deleterious affects observed
	120 ppm	45	.059	.026	
	160 ppm	45	.078	.035	
steelhead sacfry from control above	460 ppm	45	.22	.101	96-hour LC50
rainbow trout eggs	control	4	-	-	no deleterious affects observed
	300 ppm	45	.147	.066	
	400 ppm	45	.196	.088	15% mortality
rainbow trout sacfry from control above	500 ppm	45	.245	.110	96-hour LC50
rainbow trout underyearlings	51 ppm	4	.025	.011	96-hour LC50

rainbow trout *(October 1986)	1248 ppm**	50	.079	.051	96-hour LC50
rainbow trout *(February 1987)	51 ppm	4	.027	.012	96-hour LC50

* previous Ministry of Environment bioassay toxicity testing (Wilkes, 1987)

** AMD from October 1986 had metal concentrations approximately 8 times lower than in February or April 1987.